LIPOPOLYSACCHARIDE-INDUCED STIMULATION OF HUMAN MICROVASCULAR ENDOTHELIAL CELL PLASMINOGEN ACTIVATOR INHIBITOR-1 GENE EXPRESSION IS PRIMARILY REGULATED BY AN IL-6 INDUCED AUTOCRINE RESPONSE

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Endothelial cells (endos) produce both plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (PA). By regulating the levels of these proteins, endothelial cells control localized proteolytic events such as fibrinolysis in large, and small or microvascular (micro) blood vessels. It is well recognized that inflammatory stimuli such as cytokines modulate the levels of PAI-1 and PA. Interleukin-1 (IL-1) and tumor necrosis factor- (TNF-), but not interleukin-6 (IL-6) stimulate PAI-1 gene expression in large vessel endos. However, no information is known about PAI-1 regulation in micro endos. We investigated the regulation of PAI-1 gene expression in human omentum-derived micro endos by lipopolysaccharide (LPS). LPS stimulated both the production of PAI-1 and IL-6 approximately 3.0-fold within 24 hours. Treatment of micro endos with recombinant(r) human(h) IL-6 resulted in a dose-dependent three-fold increase in PAI-1 levels. As little as 5 ng/ml produced a maximal response which was equal to the level of IL-6 stimulated to LPS to produce a similar maximal PAI-1 response. This response was specific since it could be inhibited to basal levels with neutralizing antibodies to IL-6 but was unaffected by anti-human albumin. The PAI-1 response to rhIL-6 was time dependent becoming clearly significant by six hours post-treatment and reached steady-state by 10-12 hours. The LPS-induced IL-6 and PAI-1 responses were 100% and 60%, respectively, inhibited by anti-IL-6 but unaffected by anti-albumin. PAI-1 mRNA steady state levels as assessed by quantitative slot blots were increased 3.2 fold and could be inhibited by both α-amanitin and cycloheximide suggesting that *de novo* synthesis was necessary to stimulate PAI-1 mRNA transcription. It is well known in other IL-6 responsive genes such as fibrinogen that *de novo* protein synthesis of nuclear transcription factor is required for increased transcriptional activity. Nulear Run-off studies confirmed that increases in PAI-1 were due to an increased rate of transcription. These studies imply that there is a differential response to cytokines by endothelial cells derived from different site within the body. This microvascular endothelial cell response to IL-1 and IL-6 indicates that regulation of localized proteolysis particularly, fibrinolysis may be more tightly modulated than in large vessel endothelial cells.